ratios of 33 to 220. About six serial transfers were necessary to complete this adaptation.

The cultures adapted to various S/D ratios were utilized in the manufacture of Provolone cheese. For the purpose of these experiments, cultures were adapted to S/D ratios of 36, 76, 111 and 151. Two-hundred pound lots of milk were made into cheese in a 50-gallon cheese vat with an S/D ratio of 152. The data in Table 3 are representative of the effect of the S/D ratio in the bulk

TABLE 3

Effect of S/D ratio of **S. thermophilus** culture on acid development in Provolone curd[1].

Sample	Acid development with culture at following S/D ratios			
	36	76	111	151
	Percent			
Whey at cutting	.11	.11½	.11	.11
Whey at cooking	.11	.11½	.12	.12
Whey at dipping	.11	.12	.13	.14
Whey 1 hour after packing..	.12	.15	.33	.35
Whey 2 hours after packing	.13	.22	.46	.51

[1]S/D ratios of starter cultures (transferred at least six times at listed S/D before using in cheese manufacture). S/D of milk in cheese vat was 152.

starter on the development of acidity in Provolone curd. The results indicate definitely that the cause of slow acid development encountered previously was actually caused by differences in S/D ratio of the medium as compared to that of the milk in the same cheese vat. The best results were obtained when the S/D ratio of the starter was about the same as the SD ratio of the milk in the cheese vat.

TABLE 4

Effect of S/D ratio of **S. thermophilus** culture on time required for proper acid development in Provolone curd[1].

S/D ratio of starter media	Hours required from draining until pH of curd reached 5.3
36	8
54	8
76	5
111	2½
151	2
224	2

[1]S/D ratio of milk in cheese vat was 152. Average of two trials.

SUMMARY

The effect of the S/D ratio in which the culture is grown on the time required for the culture to reach a pH of 5.3 is summarized in Table 4.

Observations made in this study have shown that differences in the ratio of the surface area to the depth (S/D) of the media was related to problems involving slow acid development during the manufacture of experimental Provolone cheese. It was shown that the S/D ratio of the milk in the cheese vat must be similar to that which has been utilized in carrying the starter culture. Failure to utilize S/D ratios similar to that required in the cheese vat resulted in failure in the manufacture of Provolone cheese.

It would appear possible that some difficulties in acid production by apparently active starter cultures may be related to marked differences in surface-depth ratios of the medium in which the cultures are grown when this problem cannot be related to either bacteriophage or antibiotics.

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The effect of varying ratios of surface area to depth on the acidity development in Italian cheese starter cultures [1, 2]

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THE rapid production of acidity by starter cultures is an essential part of the cheese manufacturing process. Over the years many problems have been encountered in which lactic acid starter cultures have failed to produce acidity properly and were unsuitable for cheese manufacture. It is a common occurrence in industry to observe that cultures which produce acid rapidly in the control laboratory are unable to do so in the cheese vat. Some cheesemakers have observed that, during the cheese making operation, the acid development is less satisfactory when the vat is only partially full of milk than when the vat is filled to capacity. Generally, such failures have been attributed to antibiotics or baceriophage.

In a study being conducted to determine the effect of various starter cultures on the manufacture and ripening of Italian cheese, difficulty was encountered in using active laboratory cultures for cheese manufacturing purposes. The organisms originally had been isolated from Italian cheese or Italian cheese starter cultures and had been carried in the laboratory at the same temperatures used by the Italian cheese. All starter cultures were very active in test tube growth, being capable of producing 0.6% acidity in a six-hour incubation period. However, when these starters were used in the manufacture of Provolone cheese, it was found that from one to six hours longer than normal was required to develop sufficient acidity in the Provolone curd to permit proper molding. Examination of the cultures and of the whey from the cheese revealed the absence of and other inhibitory substances.

Attention was then directed to differences in the conditions under which the starter cultures were grown in the bulk culture flask and in the

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TABLE 1
Effect of S/D ratio on acid-producing ability of Italian cheese starter cultures[1, 2].

Incubation time	Percent acid production by following cultures														
	S. thermophilus					L. bulgaricus					L. lactis				
	S/D					S/D					S/D				
	36	76	111	151	224	36	76	111	151	224	36	76	111	151	224
0	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16
2	.17	.17	.16	.16	.16	.19	.18	.17	.17	.16	.19	.19	.16	.16	.16
4	.26	.24	.22	.20	.19	.23	.23	.19	.19	.19	.24	.23	.19	.17	.16
6	.47	.46	.40	.36	.30	.42	.44	.37	.35	.35	.44	.37	.36	.34	.32
8	.85	.82	.71	.61	.47	.75	.70	.65	.48	.48	.80	.80	.54	.48	.42
10	1.24	1.21	.91	.76	.65	1.31	1.30	1.0	.82	.81	1.26	1.20	.75	.66	.54

[1]Starters had been carried in milk media at an S/D ratio of 36 for at least six transfers prior to experiment.

[2]S/D ratio = $\frac{\text{Surface area}}{\text{Depth}}$

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cheese vat. Exploratory research indicated that the slow acid development might be related to differences in the ratio of the surface area to the depth of the milk in the culture flask as compared to the cheese vat.

The effect of variations in the surface area-depth relationship on starter activity: Cultures of *Lactobacillus lactis, Lactobacillus bulgaricus,* and *Streptococcus thermophilus,* were transferred into flasks containing different levels of milk. These were incubated at 40° C. and acidity tests were made every two hours over a 10-hour incubation period. The relationship of surface area to depth of the medium was calculated and expressed as the surface area-depth ratio (S/D). The results are shown in Table 1. The results for all three culture organisms were similar. The data revealed that these starter cultures could not produce acid rapidly when transferred into a medium with an S/D ratio of more than twice which they were generally adapted for growth.

Because of the small amount of milk required for experimental cheese manufacture, the starter cultures which were used were carried in wide-mouth culture bottles. Calculation showed an S/D ratio of 33 in the culture medium, whereas the milk in the cheese vat utilized for the manufacture of cheese had a S/D ratio of 152.

Further investigation revealed that a single transfer into a two-quart culture bottle resulted in stratification of acid-producing ability as related to the S/D relationship. Transfer was made from the top, middle and bottom of this culture flask into flasks of different S/D ratios. The results for this and subsequent experiments are reported only for *S. thermophilus.* All other cultures revealed similar results. The results are shown in Table 2. The culture transferred from the top of

TABLE 2

Acid production of **S. thermophilus** at different S/D ratios as affected by area of transfer from culture bottle.

Area of transfer	Acid production after 10 hrs. incubation at S/D ratios				
	25	50	100	150	200
			Percent		
Top S/D=150	.56	.65	0.97	1.1	0.92
Middle S/D=83	.76	.98	.96	.87	.32
Bottom S/D=20	1.02	.93	.61	.27	.24

the flask with a calculated S/D ratio of 150 did not produce acid actively at an S/D ratio below 100, but grew best at an S/D ratio comparable to that of the surface. Conversely, the culture taken from the bottom of the flask with an S/D ratio of approximately 20 would not produce acid at an S/D ratio of 150.

It was found that by progressively transferring from one flask to another with an increased S/D ratio, it was possible to adapt cultures from S/D